STN Search Results for 10/590,661 STEADMAN

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NEWS	8	JAN 28	MEDLINE and LMEDLINE reloaded with enhancements
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NEWS	14	MAR 31	IFICDB, IFIPAT, and IFIUDB enhanced with new custom IPC display formats
NEWS	15	MAR 31	CAS REGISTRY enhanced with additional experimental spectra
NEWS	16	MAR 31	CA/CAplus and CASREACT patent number format for U.S. applications updated
NEWS	17	MAR 31	LPCI now available as a replacement to LDPCI
NEWS	18	MAR 31	EMBASE, EMBAL, and LEMBASE reloaded with enhancements
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NEWS EXPRESS FEBRUARY 08 CURRENT WINDOWS VERSION IS V8.3,
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STN Search Results for 10/590,661 STEADMAN

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FILE COVERS 1907 - 9 Apr 2008 VOL 148 ISS 15
FILE LAST UPDATED: 8 Apr 2008 (20080408/ED)

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=> E HAYASHI HIROAKI/AU 25

E1	1		HAYASHI HIRIOAKI/AU
E2	1		HAYASHI HIRO O/AU
E3	555	-->	HAYASHI HIROAKI/AU
E4	6		HAYASHI HIROATSU/AU
E5	1		HAYASHI HIROBUMI/AU
E6	2		HAYASHI HIROCHIKA/AU
E7	2		HAYASHI HIROE/AU
E8	86		HAYASHI HIROFUMI/AU
E9	1		HAYASHI HIROH/AU
E10	4		HAYASHI HIROHIDE/AU
E11	21		HAYASHI HIROHITO/AU
E12	3		HAYASHI HIROICHI/AU
E13	2		HAYASHI HIROJI/AU
E14	17		HAYASHI HIROKATSU/AU
E15	1		HAYASHI HIROKAZAU/AU
E16	81		HAYASHI HIROKAZU/AU
E17	163		HAYASHI HIROKI/AU
E18	1		HAYASHI HIROKICHI/AU
E19	154		HAYASHI HIROKO/AU
E20	3		HAYASHI HIROKO K/AU
E21	26		HAYASHI HIROMASA/AU
E22	105		HAYASHI HIROMI/AU
E23	169		HAYASHI HIROMICHI/AU
E24	1		HAYASHI HIROMITI/AU
E25	134		HAYASHI HIROMITSU/AU

STN Search Results for 10/590,661 STEADMAN

```
=> S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN)
           555 "HAYASHI HIROAKI"/AU
            45 SOPHORADIOL
          9638 "TRITERPENE"
         44757 "HYDROXYLASE"
             2 "TRITERPENE HYDROXYLASE"
               ("TRITERPENE"(W)"HYDROXYLASE")
          3240 AMYRIN
L1            9 ("HAYASHI HIROAKI"/AU) AND (SOPHORADIOL OR ("TRITERPENE
HYDROXYLASE") OR AMYRIN)

=> E INOUE KENICHIRO/AU 25
E1           24      INOUE KENGO/AU
E2          318      INOUE KENICHI/AU
E3          152 --> INOUE KENICHIRO/AU
E4            2      INOUE KENICHIROU/AU
E5            1      INOUE KENICHRO/AU
E6          464      INOUE KENJI/AU
E7           31      INOUE KENJIRO/AU
E8            4      INOUE KENKICHI/AU
E9            1      INOUE KENNETH H/AU
E10           2      INOUE KENSAKU/AU
E11           3      INOUE KENSHI/AU
E12           1      INOUE KENSHU/AU
E13           7      INOUE KENSUKE/AU
E14           3      INOUE KENTA/AU
E15          55      INOUE KENTARO/AU
E16           5      INOUE KENTAROU/AU
E17           2      INOUE KENTO/AU
E18           2      INOUE KENYA/AU
E19           1      INOUE KENZIRO/AU
E20         160      INOUE KENZO/AU
E21           1      INOUE KENZOU/AU
E22           7      INOUE KIBO/AU
E23           1      INOUE KICHI/AU
E24           3      INOUE KICHIJIRO/AU
E25           1      INOUE KICHINOSUKE/AU

=> S (E3 OR E4 OR E5) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN)
           152 "INOUE KENICHIRO"/AU
             2 "INOUE KENICHIROU"/AU
             1 "INOUE KENICHRO"/AU
            45 SOPHORADIOL
          9638 "TRITERPENE"
         44757 "HYDROXYLASE"
             2 "TRITERPENE HYDROXYLASE"
               ("TRITERPENE"(W)"HYDROXYLASE")
          3240 AMYRIN
L2            7 ("INOUE KENICHIRO"/AU OR "INOUE KENICHIROU"/AU OR "INOUE
KENICHRO"/AU) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN)

=> E HOSHINO MASATERU/AU 25
E1           21      HOSHINO MASASHI/AU
E2           58      HOSHINO MASATAKA/AU
E3            1 --> HOSHINO MASATERU/AU
E4           65      HOSHINO MASATO/AU
E5           11      HOSHINO MASATOSHI/AU
E6            2      HOSHINO MASAYOSHI/AU
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STN Search Results for 10/590,661 STEADMAN

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E7           24      HOSHINO MASAYUKI/AU
E8            1      HOSHINO MASAZUMI/AU
E9            2      HOSHINO MASHAHIRO/AU
E10           2      HOSHINO MASUKI/AU
E11           5      HOSHINO MASUO/AU
E12           3      HOSHINO MAYUMI/AU
E13           3      HOSHINO MEGUMI/AU
E14           5      HOSHINO MICHIHIKO/AU
E15          36      HOSHINO MICHIO/AU
E16           4      HOSHINO MIDORI/AU
E17           3      HOSHINO MIEKO/AU
E18           3      HOSHINO MIHO/AU
E19           8      HOSHINO MIHOKO/AU
E20           4      HOSHINO MIKA/AU
E21           2      HOSHINO MIKINOBU/AU
E22         229      HOSHINO MIKIO/AU
E23           2      HOSHINO MINA/AU
E24           7      HOSHINO MINAKO/AU
E25           1      HOSHINO MINEJI/AU

=> S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN)
              1 "HOSHINO MASATERU"/AU
             45 SOPHORADIOL
           9638 "TRITERPENE"
          44757 "HYDROXYLASE"
              2 "TRITERPENE HYDROXYLASE"
                  ("TRITERPENE"(W)"HYDROXYLASE")
           3240 AMYRIN
L3            1 ("HOSHINO MASATERU"/AU) AND (SOPHORADIOL OR ("TRITERPENE
HYDROXYLASE") OR AMYRIN)

=> E SHIBUYA MASAAKI/AU 25
E1            1      SHIBUYA MAOKI/AU
E2            3      SHIBUYA MARK L/AU
E3           93 --> SHIBUYA MASAAKI/AU
E4          350      SHIBUYA MASABUMI/AU
E5            8      SHIBUYA MASACHIKA/AU
E6           24      SHIBUYA MASAFUMI/AU
E7            1      SHIBUYA MASAHARU/AU
E8            3      SHIBUYA MASAHIDE/AU
E9           55      SHIBUYA MASAHIKO/AU
E10         108      SHIBUYA MASAHIRO/AU
E11          12      SHIBUYA MASAHITO/AU
E12           1      SHIBUYA MASAJI/AU
E13           4      SHIBUYA MASAKAZU/AU
E14         137      SHIBUYA MASAKI/AU
E15          10      SHIBUYA MASAMI/AU
E16           2      SHIBUYA MASAMICHI/AU
E17           4      SHIBUYA MASANAO/AU
E18           2      SHIBUYA MASANOBU/AU
E19           2      SHIBUYA MASANORI/AU
E20          71      SHIBUYA MASAO/AU
E21          29      SHIBUYA MASAOKI/AU
E22           3      SHIBUYA MASAOMI/AU
E23           1      SHIBUYA MASARU/AU
E24          25      SHIBUYA MASASHI/AU
E25           2      SHIBUYA MASATAKA/AU

=> S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN)
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STN Search Results for 10/590,661 STEADMAN

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             93 "SHIBUYA MASAAKI"/AU
             45 SOPHORADIOL
           9638 "TRITERPENE"
          44757 "HYDROXYLASE"
              2 "TRITERPENE HYDROXYLASE"
                ("TRITERPENE"(W)"HYDROXYLASE")
           3240 AMYRIN
L4           25 ("SHIBUYA MASAAKI"/AU) AND (SOPHORADIOL OR ("TRITERPENE
HYDROXYLASE") OR AMYRIN)

=> E EBIZUKA YUTAKA/AU 25
E1            2     EBIZUKA YASUO/AU
E2            3     EBIZUKA YOSHIE/AU
E3          230 --> EBIZUKA YUTAKA/AU
E4            5     EBKE D/AU
E5            3     EBKE DANIEL/AU
E6            2     EBKE K/AU
E7            1     EBKE KLAUS/AU
E8            1     EBKE KLAUS PETER/AU
E9            1     EBKE M/AU
E10           2     EBKE T/AU
E11          10     EBKER CHRISTINA/AU
E12           7     EBLAGHIE MAXWELL C/AU
E13           1     EBLAGON F/AU
E14           1     EBLAGON FERNARDO A/AU
E15           1     EBLAN M J/AU
E16           3     EBLAN MICHAEL J/AU
E17           4     EBLE A/AU
E18           4     EBLE ALBERT F/AU
E19           1     EBLE ALBERT S/AU
E20           1     EBLE ALBERT STEPHEN/AU
E21          22     EBLE ANDREE/AU
E22           8     EBLE AXEL/AU
E23          15     EBLE B/AU
E24           1     EBLE B E/AU
E25           1     EBLE B K/AU

=> S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN)
            230 "EBIZUKA YUTAKA"/AU
             45 SOPHORADIOL
           9638 "TRITERPENE"
          44757 "HYDROXYLASE"
              2 "TRITERPENE HYDROXYLASE"
                ("TRITERPENE"(W)"HYDROXYLASE")
           3240 AMYRIN
L5           29 ("EBIZUKA YUTAKA"/AU) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE")
OR AMYRIN)

=> d his

     (FILE 'HOME' ENTERED AT 15:54:33 ON 09 APR 2008)

     FILE 'CAPLUS' ENTERED AT 15:55:43 ON 09 APR 2008
                  E HAYASHI HIROAKI/AU 25
L1             9 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN
                  E INOUE KENICHIRO/AU 25
L2             7 S (E3 OR E4 OR E5) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE
                  E HOSHINO MASATERU/AU 25
L3             1 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN
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STN Search Results for 10/590,661 STEADMAN

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                E SHIBUYA MASAAKI/AU 25
L4          25 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN
                E EBIZUKA YUTAKA/AU 25
L5          29 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN

=> s l1 or l2 or l3 or l4 or l5
L6          32 L1 OR L2 OR L3 OR L4 OR L5

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7          32 DUP REM L6 (0 DUPLICATES REMOVED)

=> d ibib abs 1-
YOU HAVE REQUESTED DATA FROM 32 ANSWERS - CONTINUE? Y/(N):y

L7   ANSWER 1 OF 32  CAPLUS  COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER:        2007:52486  CAPLUS
DOCUMENT NUMBER:         146:317068
TITLE:                   Origin of Structural Diversity in Natural Triterpenes:
                         Direct Synthesis of seco-Triterpene Skeletons by
                         Oxidosqualene Cyclase
AUTHOR(S):               Shibuya, Masaaki; Xiang, Ting; Katsube,
                         Yuji; Otsuka, Miyuki; Zhang, Hong; Ebizuka,
                         Yutaka
CORPORATE SOURCE:        Graduate School of Pharmaceutical Sciences, The
                         University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo,
                         113-0033, Japan
SOURCE:                  Journal of the American Chemical Society (2007),
                         129(5), 1450-1455
                         CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER:               American Chemical Society
DOCUMENT TYPE:           Journal
LANGUAGE:                English
OTHER SOURCE(S):         CASREACT 146:317068
GI
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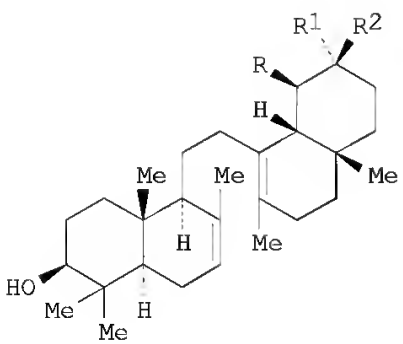


I

AB At1g78500, one of the oxidosqualene cyclase (OSC) homologues from Arabidopsis thaliana, was expressed in a lanosterol synthase-deficient yeast strain and the products were analyzed. In addition to the known triterpenes, this OSC was found to produce two new triterpenes, the structures of which were determined by NMR and MS analyses. The new

triterpenes are C-ring-seco-β- amyrin I (R = H, R1 = R2 = Me) and C-ring-seco-α- amyrin I (R = R1 = Me, R2 = H) and named β-seco- amyrin and α-seco- amyrin, resp. β-Seco- Amyrin is produced from the oleanyl cation through bond cleavage between C8 and C14, and α-seco- amyrin is produced from the ursanyl cation in the same manner. Together with Grob fragmentation catalyzed by another OSC (marneral synthase) from A. Thaliana, the formation of seco-amyrins by this OSC revealed that OSCs not only catalyze carbon-carbon bond formations and Wagner-Meerwein rearrangements but also cleave preformed ring systems in cationic intermediates. Based on this information, direct production of other natural seco-triterpenes by OSCs is proposed.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 2 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2007:736731 CAPLUS

DOCUMENT NUMBER: 147:253784

TITLE: Production of triterpene acids by cell suspension cultures of Olea europaea

AUTHOR(S): Saimaru, Hiroshi; Orihara, Yutaka; Tansakul, Pimpimon; Kang, Young-Hwa; Shibuya, Masaaki; Ebizuka, Yutaka

CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan

SOURCE: Chemical & Pharmaceutical Bulletin (2007), 55(5), 784-788
CODEN: CPBTAL; ISSN: 0009-2363

PUBLISHER: Pharmaceutical Society of Japan

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Olive (Olea europaea) contains large quantity of triterpene acids including oleanolic acid (6) as a major one. Varieties of biol. activities exhibited by triterpene acids attracted our attentions, especially from pharmaceutical viewpoints. Cell culture of olive plant was induced and its triterpene constituents were studied. From the cell suspension cultures, six ursane type triterpene acids; ursolic acid (9), pomolic acid (10), rotundic acid (11), tormentic acid (12), 2α-hydroxyursolic acid (13) and 19α-hydroxyasiatic acid (14), and two oleanane type acids; oleanolic acid and maslinic acid (7), have been isolated. Quantity of ursane type triterpene acids produced by cell cultures was larger than that of oleanane type. Further, a multifunctional oxidosqualene cyclase (OSC) named OEA was cloned by homol. based PCRs from the same cultured cells. Major product of OEA is α- amyrin (ursane skeleton), showing good accordance to higher content of ursane-type triterpene acids in the cultured cells, and strongly suggesting OEA to be a major contributor OSC for their production

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2006:995444 CAPLUS

DOCUMENT NUMBER: 146:2837

TITLE: Dammarenediol-II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in Panax ginseng

AUTHOR(S): Tansakul, Pimpimon; Shibuya, Masaaki; Kushiro, Tetsuo; Ebizuka, Yutaka

CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The

University of Tokyo, Bunkyo-ku, Tokyo, 113-0033, Japan
SOURCE: FEBS Letters (2006), 580(22), 5143-5149
CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Panax ginseng produces triterpene saponins called ginsenosides, which are classified into two groups by the skeleton of aglycons, namely dammarane type and oleanane type. Dammarane-type ginsenosides dominate over oleanane type not only in amount but also in structural varieties. However, their sapogenin structure is restricted to two aglycons, protopanaxadiol and protopanaxatriol. So far, the genes encoding oxidosqualene cyclase (OSC) responsible for formation of dammarane skeleton have not been cloned, although OSC yielding oleanane skeleton (β- amyrin synthase) has been successfully cloned from this plant. In this study, cDNA cloning of OSC producing dammmarane triterpene was attempted from hairy root cultures of P. ginseng by homol. based PCR method. A new OSC gene (named as PNA) obtained was expressed in a lanosterol synthase deficient (erg7) Saccharomyces cerevisiae strain GIL77. LC-MS and NMR analyses identified the accumulated product in the yeast transformant to be dammarenediol-II, demonstrating PNA to encode dammarenediol-II synthase.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2006:1200127 CAPLUS
DOCUMENT NUMBER: 146:94433

TITLE: Molecular cloning and functional expression of a multifunctional triterpene synthase cDNA from a mangrove species Kandelia candel (L.) Druce

AUTHOR(S): Basyuni, Mohammad; Oku, Hirosuke; Inafuku, Masashi; Baba, Shigeyuki; Iwasaki, Hironori; Oshiro, Keichiro; Okabe, Takafumi; Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24, Korimoto, Kagoshima, 890-0065, Japan

SOURCE: Phytochemistry (Elsevier) (2006), 67(23), 2517-2524
CODEN: PYTCAS; ISSN: 0031-9422
PUBLISHER: Elsevier Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Homol. based PCRs with degenerate primers designed from the conserved sequences among the known oxidosqualene cyclases (OSCs) have resulted in cloning of a triterpene synthase (KcMS) from the young roots of Kandelia candel (L.) Druce (Rhizophoraceae). KcMS consists of a 2286 bp open reading frame, which codes for 761 amino acids. The deduced amino acid sequence showed 79% homol. to a lupeol synthase from Ricinus communis suggesting it to be a lupeol synthase of K. candel. KcMS was expressed in a lanosterol synthase deficient yeast with the expression vector pYES2 under the control of GAL1 promoter. GC-MS anal. showed that the transformant accumulated a mixture of lupeol, β- amyrin and α- amyrin in a 2:1:1 ratio, indicating that KcMS encodes a multifunctional triterpene synthase, although it showed high sequence homol. to a R. communis lupeol synthase. This is the first OSC cloning from mangrove tree species.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2006:367646 CAPLUS
DOCUMENT NUMBER: 144:483346
TITLE: Identification of β- amyrin and sophoradiol 24-hydroxylase by expressed sequence tag mining and functional expression assay
AUTHOR(S): Shibuya, Masaaki; Hoshino, Masaki; Katsube, Yuji; Hayashi, Hiroaki; Kushiro, Tetsuo; Ebizuka, Yutaka
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan
SOURCE: FEBS Journal (2006), 273(5), 948-959
CODEN: FJEOAC; ISSN: 1742-464X
PUBLISHER: Blackwell Publishing Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Triterpenes exhibit a wide range of structural diversity produced by a sequence of biosynthetic reactions. Cyclization of oxidosqualene is the initial origin of structural diversity of skeletons in their biosynthesis, and subsequent regio- and stereospecific hydroxylation of the triterpene skeleton produces further structural diversity. The enzymes responsible for this hydroxylation were thought to be cytochrome P 450-dependent monooxygenase, although their cloning has not been reported. To mine these hydroxylases from cytochrome P 450 genes, five genes (CYP71D8, CYP82A2, CYP82A3, CYP82A4 and CYP93E1) reported to be elicitor-inducible genes in Glycine max expressed sequence tags (EST), were amplified by PCR, and screened for their ability to hydroxylate triterpenes (β-amyrin or sophoradiol) by heterologous expression in the yeast Saccharomyces cerevisiae. Among them, CYP93E1 transformant showed hydroxylating activity on both substrates. The products were identified as olean-12-ene-3β,24-diol and soyasapogenol B, resp., by GC-MS. Co-expression of CYP93E1 and β- amyrin synthase in S. cerevisiae yielded olean-12-ene-3β,24-diol. This is the first identification of triterpene hydroxylase cDNA from any plant species. Successful identification of a β- amyrin and sophoradiol 24-hydroxylase from the inducible family of cytochrome P 450 genes suggests that other triterpene hydroxylases belong to this family. In addition, substrate specificity with the obtained P 450 hydroxylase indicates the two possible biosynthetic routes from triterpene-monool to triterpene-triol.
REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2005:962388 CAPLUS
DOCUMENT NUMBER: 143:244075
TITLE: Preparation of soybean triterpene hydroxylase and use of the enzyme for production of soyasapogenol B
INVENTOR(S): Hayashi, Hiroaki; Inoue, Kenichiro ; Hoshino, Masateru; Shibuya, Masaaki; Ebizuka, Yutaka
PATENT ASSIGNEE(S): Meiji Seika Kaisha, Ltd., Japan
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1

STN Search Results for 10/590,661 STEADMAN

PATENT INFORMATION:

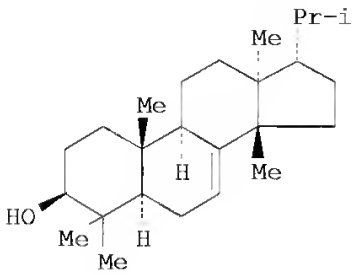
PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005080572	A1	20050901	WO 2005-JP3205	20050225
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1721983	A1	20061115	EP 2005-719556	20050225
R: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LI, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR				
US 20080003639	A1	20080103	US 2006-590661	20060825
PRIORITY APPLN. INFO.:			JP 2004-49123 A	20040225
			WO 2005-JP3205 W	20050225

AB This invention provides a process of preparation of triterpene hydroxylase from soybean. The cDNA and protein sequences of triterpene hydroxylase were disclosed. The gene encoding the triterpene hydroxylase was derived from cytochrome P 450 CYP93E1 and enzyme catalyzes the hydroxylation of oleanane triterpene at position 24. The β- amyrin synthase gene was co-expressed with triterpene for biosynthesis of soyasapogenol B.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2004:830476 CAPLUS
DOCUMENT NUMBER: 142:23399
TITLE: Enzymatic formation of an unnatural novel tetracyclic sesterterpene by β- amyrin synthase
AUTHOR(S): Noma, Hisashi; Tanaka, Hideya; Noguchi, Hiroshi; Shibuya, Masaaki; Ebizuka, Yutaka; Abe, Ikuro
CORPORATE SOURCE: School of Pharmaceutical Sciences and the 21st Century COE Program, University of Shizuoka, 52-1 Yada, Shizuoka, 422-8526, Japan
SOURCE: Tetrahedron Letters (2004), 45(45), 8299-8301
CODEN: TELEAY; ISSN: 0040-4039
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 142:23399
GI



I

AB A convergent synthesis provided a C25 and a C35 oxidopolyprene in which a farnesyl C15 unit is connected in a head-to-head fashion to a geranyl C10 or a geranylgeranyl C20 unit. When incubated with recombinant β-amyrin synthase from Pisum sativum the C25 oxidopolyprene was enzymically converted to an unnatural novel tetracyclic sesterterpene (I), while the C35 analog did not afford any cyclization product.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2004:400579 CAPLUS

DOCUMENT NUMBER: 141:106632

TITLE: Mechanism and Stereochemistry of Enzymatic Cyclization of 24,30-Bisnor-2,3-oxidosqualene by Recombinant β- Amyrin Synthase

AUTHOR(S): Abe, Ikuro; Sakano, Yuichi; Sodeyama, Megumi; Tanaka, Hideya; Noguchi, Hiroshi; Shibuya, Masaaki; Ebizuka, Yutaka

CORPORATE SOURCE: School of Pharmaceutical Sciences and the COE 21 Program, University of Shizuoka, Shizuoka, 422-8526, Japan

SOURCE: Journal of the American Chemical Society (2004), 126(22), 6880-6881
CODEN: JACSAT; ISSN: 0002-7863

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

OTHER SOURCE(S): CASREACT 141:106632

GI

* STRUCTURE DIAGRAM TOO LARGE FOR DISPLAY - AVAILABLE VIA OFFLINE PRINT *

AB Recombinant β- amyrin synthase from Pisum sativum converted 24,30-bisnor-2,3-oxidosqualene into a 3:1:0.2 mixture of 29,30-bisnor-β- amyrin (I), 29,30-bisnorgermanicol (II), and 29,30-bisnor-δ- amyrin (III). Further, enzyme reactions with [23-13C]- and [23,23-2H]-labeled isotopomers demonstrated that the cyclization did not proceed through formation of a lupanyl primary cation with a five-membered E-ring, but an electrophilic addition of the tetracyclic C-18 cation on to the terminal double bond directly generated a thermodynamically favored pentacyclic secondary cation with a less-strained six-membered E-ring. Interestingly, the formation of the three regioisomers suggested that the absence of the terminal Me groups

resulted in a structural perturbation in the folding conformation of the E-ring of the oleanyl cation at the active site of the enzyme.
REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 9 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2004:164550 CAPLUS
DOCUMENT NUMBER: 140:357519
TITLE: Enzymatic Cyclization of 22,23-Dihydro-2,3-oxidosqualene into Euph-7-en-3β-ol and Bacchar-12-en-3β-ol by Recombinant β-Amyrin Synthase
AUTHOR(S): Abe, Ikuro; Sakano, Yuichi; Tanaka, Hideya; Lou, Weiwei; Noguchi, Hiroshi; Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: School of Pharmaceutical Sciences and the COE 21 Program, University of Shizuoka, Shizuoka, 422-8526, Japan
SOURCE: Journal of the American Chemical Society (2004), 126(11), 3426-3427
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 140:357519
AB Recombinant β- amyrin synthase from Pisum sativum converted 22,23-dihydro-2,3-oxidosqualene, a substrate analog lacking the terminal double bond of 2,3-oxidosqualene, into a 4:1 mixture of euph-7-en-3β-ol and bacchar-12-en-3β-ol. This is the first demonstration of the enzymic formation of the baccharene skeleton with a six-membered D-ring. In the absence of the terminal double bond, the proton-initiated cyclization first generated the tetracyclic dammarenyl cation, followed by a backbone rearrangement with loss of H-7α leading to the formation of euph-7-en-3β-ol, while D-ring expansion to the baccharenyl cation and subsequent 1,2-hydride shifts with H-12α elimination yielded bacchar-12-en-3β-ol. It is remarkable that the formation of the anti-Markovnikov six-membered D-ring did not depend on the participation of the terminal π-electrons.
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2004:596824 CAPLUS
DOCUMENT NUMBER: 141:328561
TITLE: Differential expression of three oxidosqualene cyclase mRNAs in Glycyrrhiza glabra
AUTHOR(S): Hayashi, Hiroaki; Huang, Pengyu; Takada, Satoko; Obinata, Megumi; Inoue, Kenichiro; Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: Gifu Pharmaceutical University, Gifu, 502-8585, Japan
SOURCE: Biological & Pharmaceutical Bulletin (2004), 27(7), 1086-1092
CODEN: BPBLEO; ISSN: 0918-6158
PUBLISHER: Pharmaceutical Society of Japan
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cultured cells and intact plants of Glycyrrhiza glabra (Fabaceae) produce betulinic acid and oleanane-type triterpene saponins (soyasaponins and glycyrrhizin). To elucidate the regulation of triterpenoid

biosynthesis in G. glabra, the cDNA of lupeol synthase, an oxidosqualene cyclase (OSC) responsible for betulinic acid biosynthesis, was cloned, and expression patterns of lupeol synthase and two addnl. OSCs, β-amyrin synthase and cycloartenol synthase, were compared. The mRNA expression levels of lupeol synthase and β- amyrin synthase were consistent with the accumulation of betulinic acid and oleanane-type triterpene saponins, resp. The transcript of lupeol synthase was highly expressed in the cultured cells and root nodules. The transcript of β- amyrin synthase, an OSC responsible for oleanane-type triterpene biosynthesis, was highly expressed in the cultured cells, root nodules and germinating seeds, where soyasaponin accumulates, and in the thickened roots where glycyrrhizin accumulates. In the cultured cells, the addition of Me jasmonate up-regulated β-amyrin synthase mRNA and soyasaponin biosynthesis, but down-regulated lupeol synthase mRNA. Furthermore, the addition of gibberellin A3 down-regulated β- amyrin synthase mRNA but not lupeol synthase mRNA in the cultured cells. The mRNA levels of cycloartenol synthase, an addnl. OSC responsible for sterol biosynthesis, in the intact plant and cultured cells were relatively constant in these expts.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 11 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2003:503097 CAPLUS
DOCUMENT NUMBER: 139:288988
TITLE: Oxidosqualene cyclases from cell suspension cultures of Betula platyphylla var. japonica: Molecular evolution of oxidosqualene cyclases in higher plants
AUTHOR(S): Zhang, Hong; Shibuya, Masaaki; Yokota, Shinso; Ebizuka, Yutaka
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: Biological & Pharmaceutical Bulletin (2003), 26(5), 642-650
CODEN: BPBLEO; ISSN: 0918-6158
PUBLISHER: Pharmaceutical Society of Japan
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Betula platyphylla var. japonica is a rich source of triterpenoid as it contains dammarane type triterpenes in the leaves, and lupane type and oleanane type triterpenes in the bark. Four oxidosqualene cyclase cDNAs (BPX, BPX2, BPW and BPY) were cloned by homol. based PCR methods from cell suspension cultures of B. platyphylla var. japonica. Open reading frames consisting of 2274, 2304, 2268 and 2340 bp were ligated into yeast expression plasmid pYES2 under the control of GAL1 promoter and introduced into lanosterol synthase deficient (erg7) Saccharomyces cerevisiae strain GIL77. Analyses of in vitro enzyme activities and/or accumulated products in the transformants demonstrated that they encode cycloartenol synthase (BPX and BPX2), lupeol synthase (BPW) and β- amyrin synthase (BPY) proteins. Phylogenetic tree was constructed for all the known oxidosqualene cyclases (OSCs) including the clones obtained in this study, revealing that OSCs having the same enzyme function form resp. branches in the tree even though they derive from different plant species. Intriguing correlation was found between reaction mechanism and mol. evolution of OSCs in higher plants.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 12 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2003:321519 CAPLUS
DOCUMENT NUMBER: 139:130656
TITLE: Up-regulation of soyasaponin biosynthesis by methyl jasmonate in cultured cells of Glycyrrhiza glabra
AUTHOR(S): Hayashi, Hiroaki; Huang, Pengyu; Inoue, Kenichiro
CORPORATE SOURCE: Department of Pharmacognosy, Gifu Pharmaceutical University, Gifu, 502-8585, Japan
SOURCE: Plant and Cell Physiology (2003), 44(4), 404-411
CODEN: PCPHA5; ISSN: 0032-0781
PUBLISHER: Japanese Society of Plant Physiologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Exogenously applied Me jasmonate (MeJA) stimulated soyasaponin biosynthesis in cultured cells of Glycyrrhiza glabra (common licorice). MRNA level and enzyme activity of β- amyrin synthase (bAS), an oxidosqualene cyclase (OSC) situated at the branching point for oleanane-type triterpene saponin biosynthesis, were up-regulated by MeJA, whereas those of cycloartenol synthase, an OSC involved in sterol biosynthesis, were relatively constant Two mRNAs of squalene synthase (SQS), an enzyme common to both triterpene and sterol biosynthesis, were also up-regulated by MeJA. In addition, enzyme activity of UDP-glucuronic acid: soyasapogenol B glucuronosyltransferase, an enzyme situated at a later step of soyasaponin biosynthesis, was also up-regulated by MeJA. Accumulations of bAS and two SQS mRNAs were not transient but lasted for 7 d after exposure to MeJA, resulting in the high-level accumulation (more than 2% of dry weight cells) of soyasaponins in cultured licorice cells. In contrast, bAS and SQS mRNAs were coordinately down-regulated by yeast extract, and mRNA accumulation of polyketide reductase, an enzyme involved in 5-deoxyflavonoid biosynthesis in cultured licorice cells, was induced transiently by yeast extract and MeJA, resp.
REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 13 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2003:321359 CAPLUS
DOCUMENT NUMBER: 139:209747
TITLE: Functional genomics approach to the study of triterpene biosynthesis
AUTHOR(S): Ebizuka, Yutaka; Katsube, Yuji; Tsutsumi, Takehiko; Kushiro, Tetsuo; Shibuya, Masaaki
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: Pure and Applied Chemistry (2003), 75(2-3), 369-374
CODEN: PACHAS; ISSN: 0033-4545
PUBLISHER: International Union of Pure and Applied Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The Arabidopsis thaliana genome-sequencing project has identified the presence of 13 oxidosqualene cyclase homologs in this plant. In addition to the already identified clones, namely, CAS1 cycloartenol synthase, LUP1 lupeol synthase, and YUP8H12R.43 multifunctional triterpene synthase, two new cDNAs of the putative oxidosqualene cyclase genes, F1019.4 and T30F21.16, were obtained by polymerase chain reaction (PCR) and functionally expressed in yeast. Liquid chromatog./mass spectrometry (LC/MS) anal. led to the identification of some of their reaction products. Interestingly, except for CAS1 for sterol biosynthesis of primary metabolism, so-far-obtained all triterpene synthases of this plant are

multifunctional, producing more than one cyclization product. A feeding experiment of 13C-labeled acetate with LUP1 lupeol synthase transformant demonstrated the stereospecific water addition to lupenyl cation intermediate, yielding 3β,20-dihydroxylupane, which accounts for the multiproduct nature of this synthase.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 14 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2002:832984 CAPLUS
DOCUMENT NUMBER: 137:293692
TITLE: Process for producing soyasapogenol b
INVENTOR(S): Hayashi, Hiroaki; Inoue, Kenichiro ; Tani, Masato
PATENT ASSIGNEE(S): Meiji Seika Kaisha, Ltd., Japan
SOURCE: PCT Int. Appl., 10 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002086142	A1	20021031	WO 2002-JP3612	20020411
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
JP 2005137201	A	20050602	JP 2001-117449	20010416
AU 2002248008	A1	20021105	AU 2002-248008	20020411
PRIORITY APPLN. INFO.:			JP 2001-117449 A	20010416
			WO 2002-JP3612 W	20020411

AB Soyasapogenol B (I) is prepared from sophoradiol with plant hydroxylase. Microsome is obtained from Glycyrrhiza glabra and incubated with sophoradiol and NADPH to prepare I.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 15 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2002:563642 CAPLUS
DOCUMENT NUMBER: 137:121601
TITLE: Cloning of cDNA for isomultiflorenol synthase, a new triterpene synthase from Luffa cylindrica, involved in biosynthesis of bryonolic acid
INVENTOR(S): Hayashi, Hiroaki; Inoue, Kenichiro ; Hiraoka, Noboru; Ikeshiro, Yasumasa; Yazaki, Kazushi; Tanaka, Shigeo; Shibuya, Masaaki; Ebizuka, Yutaka
PATENT ASSIGNEE(S): Mitsui Chemicals Inc., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 13 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002209576	A	20020730	JP 2001-11612	20010119

PRIORITY APPLN. INFO.: JP 2001-11612 20010119

AB CDNA coding for isomultiflorenol synthase from Luffa cylindrica, recombinant expression, and use in biosynthetic production of plant secondary metabolites, terpenoids, in particular, are disclosed. An oxidosqualene cyclase cDNA, LcIMS1, was isolated from cultured cells of Luffa cylindrica Roem, by heterologous hybridization with cDNA of Glycyrrhiza glabra β- amyrin synthase. Expression of LcIMS1 in yeast lacking endogenous oxidosqualene cyclase activity resulted in the accumulation of isomultiflorenol, a triterpene. This is consistent with LcIMS1 encoding isomultiflorenol synthase, an oxidosqualene cyclase involved in bryonolic acid biosynthesis in cultured Luffa cells. The deduced amino-acid sequence of LcIMS1 shows relatively low identity with other triterpene synthases, suggesting that isomultiflorenol synthase should be classified into a new group of triterpene synthases. The levels of isomultiflorenol synthase and cycloartenol synthase mRNAs, which were measured with gene-specific probes, correlated with the accumulation of bryonolic acid and phytosterols over a growth cycle of the Luffa cell cultures. Isomultiflorenol synthase mRNA was low during the early stages of cell growth and accumulated to relatively high levels in the late stages. Induction of this mRNA preceded accumulation of bryonolic acid. In contrast, cycloartenol synthase mRNA accumulated in the early stages of the culture cycle, whereas phytosterols accumulated at the same relative rate throughout the whole growth cycle. These results suggest independent regulation of these two genes and of the accumulation of bryonolic acid and phytosterols.

L7 ANSWER 16 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2002:424071 CAPLUS
DOCUMENT NUMBER: 136:397651
TITLE: Challenge to produce "unnatural" triterpenes
AUTHOR(S): Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: Grad. Sch. Pharm. Sci., The Univ. Tokyo, Tokyo, 113-0033, Japan
SOURCE: Baiosaiensu to Indasutori (2002), 60(5), 314-315
CODEN: BIDSE6; ISSN: 0914-8981
PUBLISHER: Baioindasutori Kyokai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese

AB A review on the structure of triterpenes and sterols, discovery of oxidosqualene cyclases, site-directed mutagenesis of lupeol synthase into β- amyrin synthase, and production of novel unnatural triterpenes by mutant enzymes. A mutant lupeol synthase (Leu256Trp) produced exclusively β- amyrin with only minor amount of lupeol. A mutant β- amyrin synthase (Tyr261His) produced novel terpenes.

L7 ANSWER 17 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2002:182571 CAPLUS
DOCUMENT NUMBER: 136:306693
TITLE: Biosynthesis of triterpenes in higher plants: towards production of "unnatural" triterpenes
AUTHOR(S): Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: Graduate School of Pharmacy, University of Tokyo,

Japan
SOURCE: Yuki Gosei Kagaku Kyokaishi (2002), 60(3), 195-205
CODEN: YGKKAE; ISSN: 0037-9980
PUBLISHER: Yuki Gosei Kagaku Kyokai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese

AB A review. Cyclization of oxido-squalene into tetra- and pentacyclic carbon skeleton of sterols and triterpenes, catalyzed by oxido-squalene cyclases(OSCs), is one of the most complex and fascinating reactions found in nature. OSCs generate multiple stereogenic centers in a single reaction, and are responsible for the diverse triterpene skeletons. In order to investigate the origin of structural diversity of triterpene skeletons, cDNA cloning of OSCs and anal. of their product specificity were carried out. From triterpene producing plants, over twenty-five OSC clones were obtained, and their enzyme function established by expression in yeast. They included cycloartenol, cucurbitadienol, lupeol, P-amyrin, isomultiflorenol and mixed amyrin synthases.
Studies of chimeric proteins between,B-amyrin synthase and lupeol synthase, and mutant proteins constructed by site directed mutagenesis identified the amino acid residues responsible for their product specificity. Trp 259 of P-amyrin synthase (PNY) was identified to be the critical residue controlling β- amyrin formation. In further mutation studies, PNY Y 261 H mutant produced dammara-18,21-dien-3β,-ol (as a 3:5 mixture of E/Z isomer at Δ18) together with minor amount of dammara-18(28),21-dien-3β-ol. These triterpenes have not been reported from nature, and therefore, could be categorized as "unnatural" natural products. The results of this study opened up the possibility of generating new triterpene synthases with addnl. novel functions through point mutations.

L7 ANSWER 18 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2001:906044 CAPLUS
DOCUMENT NUMBER: 136:130599

TITLE: Molecular cloning and characterization of isomultiflorenol synthase, a new triterpene synthase from Luffa cylindrica, involved in biosynthesis of bryonolic acid

AUTHOR(S): Hayashi, Hiroaki; Huang, Pengyu; Inoue, Kenichiro; Hiraoka, Noboru; Ikeshiro, Yasumasa; Yazaki, Kazufumi; Tanaka, Shigeo; Kushiro, Tetsuo; Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: Gifu Pharmaceutical University, Gifu, 502-8585, Japan

SOURCE: European Journal of Biochemistry (2001), 268(23), 6311-6317
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An oxidosqualene cyclase cDNA, LcIMS1, was isolated from cultured cells of Luffa cylindrica Roem, by heterologous hybridization with cDNA of Glycyrrhiza glabra β- amyrin synthase. Expression of LcIMS1 in yeast lacking endogenous oxidosqualene cyclase activity resulted in the accumulation of isomultiflorenol, a triterpene. This is consistent with LcIMS1 encoding isomultiflorenol synthase, an oxidosqualene cyclase involved in bryonolic acid biosynthesis in cultured Luffa cells. The deduced amino-acid sequence of LcIMS1 shows relatively low identity with other triterpene synthases, suggesting that isomultiflorenol synthase should be classified into a new group of triterpene synthases. The levels of isomultiflorenol synthase and cycloartenol synthase mRNAs, which were

measured with gene-specific probes, correlated with the accumulation of bryonolic acid and phytosterols over a growth cycle of the Luffa cell cultures. Isomultiflorenol synthase mRNA was low during the early stages of cell growth and accumulated to relatively high levels in the late stages. Induction of this mRNA preceded accumulation of bryonolic acid. In contrast, cycloartenol synthase mRNA accumulated in the early stages of the culture cycle, whereas phytosterols accumulated at the same relative rate throughout the whole growth cycle. These results suggest independent regulation of these two genes and of the accumulation of bryonolic acid and phytosterols.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 19 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2001:584526 CAPLUS
DOCUMENT NUMBER: 135:238562
TITLE: Cloning and characterization of a cDNA encoding β- amyrin synthase involved in glycyrrhizin and soyasaponin biosyntheses in licorice
AUTHOR(S): Hayashi, Hiroaki; Huang, Pengyu; Kirakosyan, Ara; Inoue, Kenichiro; Hiraoka, Noboru; Ikeshiro, Yasumasa; Kushiro, Tetsuo; Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: Gifu Pharmaceutical University, Gifu, 502-8585, Japan
SOURCE: Biological & Pharmaceutical Bulletin (2001), 24(8), 912-916
CODEN: BPBLEO; ISSN: 0918-6158
PUBLISHER: Pharmaceutical Society of Japan
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An oxidosqualene cyclase cDNA, termed GgbAS1, was isolated from cultured cells of licorice (Glycyrrhiza glabra) by heterologous hybridization with cDNA of Arabidopsis thaliana LUP1 lupeol synthase. The yeast transformed with an expression vector containing the open reading frame of GgbAS1 produced β- amyrin, indicating that GgbAS1 encodes β- amyrin synthase involved in the glycyrrhizin and soyasaponin biosyntheses in licorice. Northern blot anal. showed that the level of β- amyrin synthase mRNA was drastically changed in the cultured licorice cells, whereas the mRNA level of cycloartenol synthase was relatively constant

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 20 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2001:540656 CAPLUS
DOCUMENT NUMBER: 135:118535
TITLE: Science of diversity: natural products science
AUTHOR(S): Ebizuka, Yutaka
CORPORATE SOURCE: Grad. Sch. Pharm., The Univ. Tokyo, Japan
SOURCE: Farumashia (2001), 37(7), 607-612
CODEN: FARUAW; ISSN: 0014-8601
PUBLISHER: Pharmaceutical Society of Japan
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese

AB A review with 22 refs., on (1) diversity in the second metabolites of microorganisms and plants, (2) X-ray crystal structure, substrate specificity, and reaction products of chalcone synthase superfamily members, (3) phylogenetic tree of oxidosqualene cyclases of plants, (4) search for the functional sites of β- amyrin synthase,

lupeol synthase, and other triterpene synthases, and (5) functional anal. of fungal polyketide synthases.

L7 ANSWER 21 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2001:412377 CAPLUS
DOCUMENT NUMBER: 136:34651
TITLE: Biosynthesis of sterols and triterpenes in higher plants
AUTHOR(S): Shibuya, Masaaki
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Bunkyo-ku, Hongo, 113-0033, Japan
SOURCE: Natural Medicines (Tokyo, Japan) (2001), 55(1), 1-6
CODEN: NMEDEO; ISSN: 1340-3443
PUBLISHER: Japanese Society of Pharmacognosy
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review. Cyclization of oxidosqualene into tetra- and pentacyclic carbon skeleton of sterols and triterpenes is one of the most complex and fascinating reactions found in nature which are catalyzed by oxidosqualene cyclases (OSCs). In order to obtain insights in mol. evolution of triterpene synthases and their catalytic mechanisms, cDNA cloning of triterpene synthases from Panax ginseng, Olea europaea, Taraxacum officinale, Betula platyphylla, Cucurbita pepo, Glycyrrhiza glabra, Luffa cylindrica, Pisum sativum and Allium macrostemon was conducted. From those nine plant materials, twenty-four OSC clones in total were obtained, sixteen of which were identified as cycloartenol, cucurbitadienol, lupeol, β- amyrin and mixed amyrin synthases.
Phylogenetic tree anal. revealed that OSCs, having the same enzyme function, formed a branch in the tree even though they had been derived from different plant species. This mode of mol. evolution is characteristic of triterpene cyclases, which is not recognized in mono-, sesqui- and diterpene cyclases. An intriguing correlation was found between the reaction mechanism and the mol. evolution of OSCs in higher plants.

L7 ANSWER 22 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 2000:461407 CAPLUS
DOCUMENT NUMBER: 133:234383
TITLE: Mutational Studies on Triterpene Synthases: Engineering Lupeol Synthase into β- Amyrin Synthase
AUTHOR(S): Kushiro, Tetsuo; Shibuya, Masaaki; Masuda, Kazuo; Ebizuka, Yutaka
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: Journal of the American Chemical Society (2000), 122(29), 6816-6824
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Site-directed mutagenesis was carried out on two triterpene synthases, β- amyrin (PNY) and lupeol (OEW) synthases, to identify the amino acid residues responsible for their product specificity. In addition to sequence comparison among known oxidosqualene cyclases, our previous chimeric studies suggested that 258MWCYCR263 sequence of β- amyrin synthase PNY (255MLCYCR260 sequence of lupeol synthase OEW) would participate in product differentiation. To test this hypothesis, Trp259 (MWCYCR of PNY) was mutated to Leu (PNY W259L mutant). Functional

expression in yeast and product anal. revealed that this mutant produced lupeol as a major product together with β- amyrin in 2:1 ratio. Some other minor products including butyrospermol were also produced. On the other hand, Leu256 (MLCYCR of OEW) was mutated to Trp (OEW L256W mutant). This mutant produced exclusively β- amyrin with only minor amount of lupeol, demonstrating that a single mutation had engineered lupeol synthase into β- amyrin synthase. Therefore, Trp259 of β- amyrin synthase was identified to be the residue controlling β- amyrin formation presumably through stabilization of oleanyl cation, while lack of this effect by Leu residue may terminate the reaction at lupenyl cation stage. In further mutation studies, Tyr residue (MWCYCR in PNY and MLCYCR in OEW) conserved in all of the OSCs producing pentacyclic triterpenes was mutated into His which is found in all of those producing tetracyclic carbon skeletons to investigate the role of this Tyr261 of PNY. PNY Y261H mutant produced dammara-18,21-dien-3β-ol (as a 3:5 mixture of E/Z isomer at Δ18) together with a minor amount of dammara-18(28),21-dien-3β-ol, demonstrating that Tyr261 of β- amyrin synthase plays an important role in producing pentacyclic triterpenes presumably by stabilizing one of the cation intermediates generated after dammarenyl cation.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 23 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2000:446342 CAPLUS

DOCUMENT NUMBER: 133:234256

TITLE: Molecular cloning and functional expression of triterpene synthases from pea (Pisum sativum) new α- amyrin-producing enzyme is a multifunctional triterpene synthase

AUTHOR(S): Morita, Masayo; Shibuya, Masaaki; Kushiro, Tetsuo; Masuda, Kazuo; Ebizuka, Yutaka

CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan

SOURCE: European Journal of Biochemistry (2000), 267(12), 3453-3460
CODEN: EJBCAI; ISSN: 0014-2956

PUBLISHER: Blackwell Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Ursane type triterpene is one of the most widespread triterpene aglycons found in plants, together with oleanane type, and these two types often occur together in the same plant. Pisum sativum is known to produce both types of triterpenes. Homol. based PCRs with degenerate primers designed from the conserved sequences found in the known β- amyrin synthases have resulted in cloning of two triterpene synthase cDNAs from immature seeds of P. sativum. They show high sequence identities to each other (78%) and also to the known β- amyrin synthases (70-90%). ORFs of the full-length clones named as PSY (2277 bp, codes for 759 amino acids) and PSM (2295 bp, codes for 765 amino acids) were ligated into the yeast expression vector pYES2 under the control of GAL1 promoter. Heterologous expression in yeast revealed PSY to be a P. sativum β- amyrin synthase. Surprisingly, however, PSM turned out to be a novel mixed amyrin synthase producing both α- and β- amyrin. Several minor triterpenes were also identified as the PSM byproducts. The presence of such multifunctional triterpene synthase would account for the co-occurrence of ursane and oleanane type triterpenes in plants.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 24 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1999:73188 CAPLUS
DOCUMENT NUMBER: 130:263975
TITLE: Chimeric Triterpene Synthase. A Possible Model for Multifunctional Triterpene Synthase
AUTHOR(S): Kushiro, Tetsuo; Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: Journal of the American Chemical Society (1999), 121(6), 1208-1216
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two triterpene synthases, β- amyrin synthase (EC 5.4.99.-) from Panax ginseng and lupeol synthase (EC 5.4.99.-) from Arabidopsis thaliana, were used to construct a series of chimeric proteins between these two enzymes in order to investigate the region important for product specificity. Functional expression in yeast and anal. of the synthase products have revealed that chimera 1, in which the N-terminal half is β- amyrin synthase and the C-terminal half is lupeol synthase, produced both β- amyrin and lupeol in a 3:1 ratio. By dividing the whole sequence into four regions, all the possible combinations of the two synthases were constructed. Among them, chimera 7, in which only region B (the second quarter from the N-terminus) is β- amyrin synthase, produced β- amyrin and lupeol in a 4:1 ratio, indicating the importance of region B in β- amyrin formation. Another chimera, which was created by the mixed PCR method, produced β- amyrin and lupeol in a 1:4 ratio, indicating that the sequence which is important for product distribution lies within 80 amino acid residues in region B. The incorporation experiment of [1,2-13C2]acetate showed that, during the formation of lupeol, the final proton abstraction takes place from either of the two gem-di-Me groups in a 1:1 ratio. This is the first demonstration of the scrambling of Me groups during the biosynthesis of any terpenoids. On the other hand, no scrambling of Me groups was observed during β- amyrin formation, indicating that the iso-Pr group of the lupenyl cation must be held tightly by β- amyrin synthase protein.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 25 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1999:779015 CAPLUS
DOCUMENT NUMBER: 132:261920
TITLE: Biosynthesis of sterols and triterpenes in higher plants: molecular evolution of triterpene synthases
AUTHOR(S): Shibuya, Masaaki; Kushiro, Tetsuo; Zhang, Hong; Morita, Masayo; Adachi, Shinya; Ebizuka, Yutaka; Hayashi, Hiroaki; Yokota, Shinso
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan
SOURCE: Tennen Yuki Kagobutsu Toronkai Koen Yoshishu (1999), 41st, 445-450
CODEN: TYKYDS

PUBLISHER: Nippon Kagakkai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review (discussion) with no refs. The cyclizations of oxidosqualene into tetra- and pentacyclic carbon skeleton of sterols and triterpenes are one of the most complex and fascinating reactions found in nature and are catalyzed by enzymes termed as oxidosqualene cyclases (OSCs). In order to obtain insights in mol. evolution of triterpene synthases and their catalytic mechanisms, cDNA cloning of triterpene synthases from Panax ginseng, Olea europaea, Taraxacum officinale, Betula platyphylla, Cucurbita pepo, Glycyrrhiza glabra, Luffa cylindrica, Pisum sativum and Allium macrostemon, was conducted. Homol. based PCR method was attempted to obtain the cDNA of OSCs. So far, 24 clones were obtained from the above plants. To determine the enzyme functions of the translation products, they were expressed in ERG7 deficient yeast mutant. Accumulation of β- amyrin (clone Y), lupeol (clone W) or cucurbitadienol (clone Q) in the cells of yeast transformants proved clone Y, W and Q to encode β- amyrin synthase, lupeol synthase and cucurbitadienol synthase proteins, resp. In order to clarify the evolutional relationships among plant OSCs, sequence homologies between all the cloned plant OSCs have been calculated and a phylogenetic tree constructed. Cycloartenol synthase clones form one big cluster in a phylogenetic tree, clearly demonstrating that plants have acquired cycloartenol synthase gene before diverged into individual species during the course of evolution, and that triterpene synthase genes have evolved from cycloartenol synthase genes. Lupeol and β- amyrin synthases from various plants also form an each cluster resp. in the tree. It is very interesting to note that unlike mono-, sesqui- and diterpene cyclases, plant triterpene synthases show correlation between mol. evolution and reaction mechanism.

L7 ANSWER 26 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1999:742473 CAPLUS
DOCUMENT NUMBER: 132:105432
TITLE: Two branches of the lupeol synthase gene in the molecular evolution of plant oxidosqualene cyclases
AUTHOR(S): Shibuya, Masaaki; Zhang, Hong; Endo, Aki; Shishikura, Kaori; Kushiro, Tetsuo; Ebizuka, Yutaka
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: European Journal of Biochemistry (1999), 266(1), 302-307
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two new triterpene synthase cDNAs, named as OEW and TRW, were cloned from olive leaves (Olea europaea) and from dandelion roots (Taraxacum officinale), resp., by the PCR method with primers designed from the conserved sequences found in the known oxidosqualene cyclases. Their ORFs consisted of 2274 bp nucleotides and coded for 758 amino acid long polypeptides. They shared high sequence identity (78%) to each other, while they showed only about 60% identities to the known triterpene synthases LUPI (lupeol synthase clone from Arabidopsis thaliana) and PNY (β- amyrin synthase clone from Panax ginseng) at amino acid level. To determine the enzyme functions of the translates, they were expressed in an ERG7 deficient yeast mutant. Accumulation of lupeol in the cells of yeast transformants proved both of these clones code for

lupeol synthase proteins. An EST (expression sequence tag) clone isolated from Medicago truncatula roots as a homolog of cycloartenol synthase gene, exhibits high sequence identity (75-77%) to these two lupeol synthase cDNAs, suggesting it to be another lupeol synthase clone. Comparatively low identity (≈ 57%) of LUP1 from Arabidopsis thaliana to either one of these clones leaves LUP1 as a distinct clone among lupeol synthases. From these sequence comparisons, now we propose that two branches of lupeol synthase gene have been generated in higher plants during the course of evolution.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 27 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 1999:776292 CAPLUS

DOCUMENT NUMBER: 132:233334

TITLE: Structure and function of triterpene synthases; mechanistic studies on the product specificities exhibited by β- amyrin and lupeol synthases

AUTHOR(S): Kushiro, Tetsuo; Shibuya, Masaaki; Ebizuka, Yutaka; Masuda, Kazuo

CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan

SOURCE: Tennen Yuki Kagobutsu Toronkai Koen Yoshishu (1999), 41st, 193-198
CODEN: TYKYDS

PUBLISHER: Nippon Kagakkai

DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese

AB A discussion and review with 6 refs. on the authors' works. Triterpenoids are one of the most abundant natural products commonly occurring in plants and exhibit a wide range of structural diversity. These triterpene frameworks are believed to be biosynthesized from a common precursor 2,3-oxidosqualene by distinct triterpene synthases. In order to identify the origin of the product specificities exhibited by triterpene synthases, we have chosen β- amyrin synthase (PNY) and lupeol synthases (LUP1, OEW) for mechanistic studies. The cyclization mechanisms leading to β- amyrin and lupeol are identical up to lupenyl cation stage where proton abstraction from the Me group results in lupeol while ring expansion and hydride shift will generate β- amyrin. To determine the polypeptide region important for the product specificity, several chimeric enzymes were constructed. Chimera 1, in which N-terminal half is PNY and C-terminal half is LUPI, produced both β- amyrin and lupeol in 3:1 ratio. In addition, minor amount of butyrospermol was produced. The results from other chimeric enzymes indicated that the 80 amino acid sequence located in the second quarter from N-terminus was important for β- amyrin formation. [1,2-13C2]Acetate feeding experiment was conducted to identify from which Me group is proton abstracted during lupeol formation. The result from LUPI showed that the proton is abstracted from both Me groups in non-specific manner. On the other hand, OEW exhibited specific proton abstraction from (Z)-Me group of 2,3-oxidosqualene. These results suggested the occurrence of two types of lupeol synthases in nature. Furthermore, site-directed mutagenesis was carried out in order to define the amino acid residue responsible for product specificity. PNY W259L mutant gave significant amount of lupeol together with 3-amyrin, while OEW L258W mutant gave exclusively β- amyrin. PNY Y261H mutant gave neither β- amyrin nor lupeol and instead it produced mixture of 5 and 6. These results suggested that Trp 259 of PNY stabilizes the oleanyl

cation during β- amyrin formation, while Tyr 261 is responsible for the formation of pentacyclic triterpenes.

L7 ANSWER 28 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1998:574614 CAPLUS
DOCUMENT NUMBER: 129:327622
TITLE: β- Amyrin synthase. Cloning of oxidosqualene cyclase that catalyzes the formation of the most popular triterpene among higher plants
AUTHOR(S): Kushiro, Tetsuo; Shibuya, Masaaki; Ebizuka, Yutaka
CORPORATE SOURCE: The Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan
SOURCE: European Journal of Biochemistry (1998), 256(1), 238-244
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB β- Amyrin, a typical pentacyclic triterpene having an oleanane skeleton, is one of the most commonly occurring triterpenes in nature and is biosynthesized from (3S)-2,3-oxidosqualene. The enzyme, β- amyrin synthase, catalyzing the cyclization of oxidosqualene into β- amyrin, generates five rings and eight asym. centers in a single transformation. A homol.-based PCR method was attempted to obtain the cDNA of this enzyme from the hairy root of Panax ginseng which produces oleanane saponins together with dammarane-type saponins. Two sets of degenerate oligonucleotide primers were designed at the regions which are highly conserved among known oxidosqualene cyclases (OSCs). Nested PCRs using these primers successfully amplified the core fragment which revealed the presence of two OSC clones PNX and PNY. Specific amplification of each clone by 3'-RACE and 5'-RACE was carried out to obtain the whole sequences. The two clones exhibited 60% amino acid identity to each other. A full-length clone of PNY was ligated into the yeast expression vector pYES2 under the GALI promoter to give pOSCPNY. β- Amyrin production was observed with the mutant yeast lacking lanosterol synthase, transformed by this plasmid. The sequence of pOSCPNY contains an open reading frame of 2289 nucleotides which codes for 763 amino acids with a predicted mol. mass of 88 kDa. Sequence comparison with other OSCs showed a high level of similarity with lanosterol, cycloartenol and lupeol synthases. The other clone, pOSCPNX, was shown to be cycloartenol synthase by similar expression in yeast. The present studies have revealed that distinct OSC exists for triterpene formation in higher plants, and the high level of similarity with cycloartenol synthase indicates close evolutionary relationship between sterol and triterpene biosynthesis.
REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 29 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1993:96774 CAPLUS
DOCUMENT NUMBER: 118:96774
TITLE: Purification and properties of squalene-2,3-epoxide cyclases from pea seedlings
AUTHOR(S): Abe, Ikuro; Sankawa, Ushio; Ebizuka, Yutaka
CORPORATE SOURCE: Fac. Pharm. Sci., Univ. Tokyo, Tokyo, 113, Japan
SOURCE: Chemical & Pharmaceutical Bulletin (1992), 40(7), 1755-60
CODEN: CPBTAL; ISSN: 0009-2363

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Dramatic changes in the activities of squalene-2,3-epoxide: cycloartenol cyclase and β- amyrin cyclase were observed in germinating pea seeds. By taking advantage of this phenomenon, the two cyclases were purified from pea seedlings. The cyclases were purified to homogeneity by solubilization with Triton X-100, chromatog. on hydroxylapatite and diethylaminoethyl (DEAE)-cellulose, isoelec. focusing and gel filtration. Cycloartenol cyclase was purified 471-fold to a specific activity of 167 pkat/mg protein, while β- amyrin cyclase was purified 4290-fold to a specific activity of 28 pkat/mg protein. They each showed a single band on sodium dodecyl sulfate polyacrylamide gel electrophoresis with a mol. mass of 55 and 35 kilodaltons (kDa), resp. The apparent Km values for (3S)-squalene-2,3-epoxide were estimated to be 25 and 50 μM, resp. The cyclases required Triton X-100 or deoxycholate for their highest activity and each showed a broad pH optimum within the range of pH 6.5 - 7.5. Inhibition by p-chloromercuribenzene sulfonic acid and N-ethylmaleimide suggested involvement of an SH group at the active site of each enzyme.

L7 ANSWER 30 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1989:419825 CAPLUS
DOCUMENT NUMBER: 111:19825
TITLE: Purification of 2,3-oxidosqualene:β-amyrin cyclase from pea seedlings
AUTHOR(S): Abe, Ikuro; Sankawa, Ushio; Ebizuka, Yutaka
CORPORATE SOURCE: Fac. Pharm. Sci., Univ. Tokyo, Tokyo, 113, Japan
SOURCE: Chemical & Pharmaceutical Bulletin (1989), 37(2), 536-8
CODEN: CPBTAL; ISSN: 0009-2363
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 2,3-Oxidosqualene:β- amyrin cyclase (E.C.5.4.99) was purified from pea seedlings in 8 steps as a soluble and homogeneous enzyme. The purified enzyme showed a single band in SDS-PAGE with a mol. weight of 35 KDa and had a Km value of 50 μM. The β- amyrin cyclase had a different mol. weight from that of cycloartenol cyclase, and these 2 enzymes were responsible for the dramatic alteration in triterpenoid and steroid biosynthesis during germination.

L7 ANSWER 31 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1990:72655 CAPLUS
DOCUMENT NUMBER: 112:72655
TITLE: Purification of squalene-2,3-epoxide cyclases from cell suspension cultures of Rabdosia japonica Hara
AUTHOR(S): Abe, Ikuro; Ebizuka, Yutaka; Seo, Shujiro; Sankawa, Ushio
CORPORATE SOURCE: Fac. Pharm. Sci., Univ. Tokyo, Tokyo, 113, Japan
SOURCE: FEBS Letters (1989), 249(1), 100-4
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Microsomes prepared from cell suspension cultures of R. japonica Hara showed activities for cyclizing squalene 2,3-epoxide into cycloartenol, β-amyrin, and α- amyrin in the presence of Triton X-100. These activities were efficiently solubilized by treatment with Triton X-100 and separated by chromatog. on hydroxylapatite, DEAE-cellulose, isoelec. focusing, and gel filtration. The purified cycloartenol cyclase showed a single band on SDS-PAGE electrophoresis with a mol. weight of

54,000, whereas β- amyrin cyclase gave a single band with a mol. weight of 28,000.

L7 ANSWER 32 OF 32 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1989:188225 CAPLUS
DOCUMENT NUMBER: 110:188225
ORIGINAL REFERENCE NO.: 110:31139a,31142a
TITLE: Purification of 2,3-oxidosqualene:cycloartenol cyclase from pea seedlings
AUTHOR(S): Abe, Ikuro; Ebizuka, Yutaka; Sankawa, Ushio
CORPORATE SOURCE: Fac. Pharm. Sci., Univ. Tokyo, Tokyo, 113, Japan
SOURCE: Chemical & Pharmaceutical Bulletin (1988), 36(12), 5031-4
CODEN: CPBTAL; ISSN: 0009-2363
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Membrane-bound 2,3-oxidosqualene-cycloartenol cyclase (EC 5.4.99.8) (I) was purified 471-fold from pea seedlings in 6 steps as a soluble and homogeneous enzyme with a yield of 10%. Purified I showed a single band in SDS-PAGE with a mol. weight of 55,000 and had a Km of 25 μM for 2,3-oxidosqualene. I required Triton X-100 or deoxycholate for its highest activity. The time course changes of I and 2,3-oxidosqualene-β- amyrin cyclase (II) in pea seedlings after germination were also determined II activity was maximum on the 3rd day after germination and fell rapidly to <10% of its maximum by the 5th day, whereas the activity of I reached its maximum on the 4th day after germination and then fell to 1/3 of the maximum and maintained the same level.

=>
=> file .djs scisearch

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FULL ESTIMATED COST	167.16	167.58

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FILE 'SCISEARCH' ENTERED AT 16:16:54 ON 09 APR 2008

STN Search Results for 10/590,661 STEADMAN


```
=> s 24-hydroxylase OR "triterpene hydroxylase" OR (24-position and hydroxylase) OR
(amyrin (w) hydroxylase) OR "sophoradiol hydroxylase" OR (amyrin (w)
24-hydroxylase) OR "sophoradiol 24-hydroxylase"
L8         3865 24-HYDROXYLASE OR "TRITERPENE HYDROXYLASE" OR (24-POSITION AND
                HYDROXYLASE) OR (AMYRIN (W) HYDROXYLASE) OR "SOPHORADIOL HYDROXY
                LASE" OR (AMYRIN (W) 24-HYDROXYLASE) OR "SOPHORADIOL 24-HYDROXYL
                ASE"

=> s 24-hydroxylase OR "triterpene hydroxylase" OR (amyrin (w) hydroxylase) OR
"sophoradiol hydroxylase" OR (amyrin (w) 24-hydroxylase) OR "sophoradiol
24-hydroxylase"
L9         3862 24-HYDROXYLASE OR "TRITERPENE HYDROXYLASE" OR (AMYRIN (W) HYDROX
                YLASE) OR "SOPHORADIOL HYDROXYLASE" OR (AMYRIN (W) 24-HYDROXYLAS
                E) OR "SOPHORADIOL 24-HYDROXYLASE"

=> l8 AND (amyrin OR sophoradiol)
L8 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l8 AND (amyrin OR sophoradiol)
L10           7 L8 AND (AMYRIN OR SOPHORADIOL)

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11           3 DUP REM L10 (4 DUPLICATES REMOVED)

=> s l11 not l7
L12           2 L11 NOT L7

=> d ibib abs 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L12  ANSWER 1 OF 2     MEDLINE on STN
ACCESSION NUMBER:    2006092364     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 16478469
TITLE:               Identification of beta-amyrin and
                     sophoradiol 24-hydroxylase by
                     expressed sequence tag mining and functional expression
                     assay.
AUTHOR:              Shibuya Masaaki; Hoshino Masaki; Katsube Yuji; Hayashi
                     Hiroaki; Kushiro Tetsuo; Ebizuka Yutaka
CORPORATE SOURCE:    Graduate School of Pharmaceutical Sciences, The University
                     of Tokyo, Japan.
SOURCE:              The FEBS journal, (2006 Mar) Vol. 273, No. 5, pp. 948-59.
                     Journal code: 101229646. ISSN: 1742-464X.
PUB. COUNTRY:        England: United Kingdom
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
                     (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200604
ENTRY DATE:          Entered STN: 16 Feb 2006
                     Last Updated on STN: 6 Apr 2006
                     Entered Medline: 5 Apr 2006
AB   Triterpenes exhibit a wide range of structural diversity produced by a
```

seguence of biosynthetic reactions. Cyclization of oxidosqualene is the initial origin of structural diversity of skeletons in their biosynthesis, and subseguent regio- and stereospecific hydroxylation of the triterpene skeleton produces further structural diversity. The enzymes responsible for this hydroxylation were thought to be cytochrome P450-dependent monooxygenase, although their cloning has not been reported. To mine these hydroxylases from cytochrome P450 genes, five genes (CYP71D8, CYP82A2, CYP82A3, CYP82A4 and CYP93E1) reported to be elicitor-inducible genes in Glycine max expressed sequence tags (EST), were amplified by PCR, and screened for their ability to hydroxylate triterpenes (beta-amyrin or sophoradiol) by heterologous expression in the yeast Saccharomyces cerevisiae. Among them, CYP93E1 transformant showed hydroxylating activity on both substrates. The products were identified as olean-12-ene-3beta,24-diol and soyasapogenol B, respectively, by GC-MS. Co-expression of CYP93E1 and beta-amyrin synthase in S. cerevisiae yielded olean-12-ene-3beta,24-diol. This is the first identification of triterpene hydroxylase cDNA from any plant species. Successful identification of a beta-amyrin and sophoradiol 24-hydroxylase from the inducible family of cytochrome P450 genes suggests that other triterpene hydroxylases belong to this family. In addition, substrate specificity with the obtained P450 hydroxylase indicates the two possible biosynthetic routes from triterpene-monool to triterpene-triol.

L12 ANSWER 2 OF 2 WPIDS COPYRIGHT 2008 THE THOMSON CORP on STN
ACCESSION NUMBER: 2002-750826 [81] WPIDS
DOC. NO. CPI: C2002-212873 [81]
TITLE: Production of soyapogenol B by treating sophoradiol with plant-originated hydroxylase, for use in drugs and pharmaceutical raw materials in treating e.g. thrombosis and tumor or protecting liver
DERWENT CLASS: B01; D16
INVENTOR: HAYASHI H; INOUE K; TANI M
PATENT ASSIGNEE: (MEIJ-C) MEIJI SEIKA KAISHA LTD
COUNTRY COUNT: 98

PATENT INFO ABBR.:

PATENT NO	KIND	DATE	WEEK	LA	PG	MAIN IPC
WO 2002086142	A1	20021031	(200281)*	JA	10[0]	
AU 2002248008	A1	20021105	(200433)	EN		
JP 2005137201	A	20050602	(200537)	JA	4	

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002086142	A1	WO 2002-JP3612	20020411
JP 2005137201	A	JP 2001-117449	20010416
AU 2002248008	A1	AU 2002-248008	20020411

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2002248008	A1	Based on	WO 2002086142 A

STN Search Results for 10/590,661 STEADMAN

PRIORITY APPLN. INFO: JP 2001-117449 20010416
AN 2002-750826 [81] WPIDS
AB WO 2002086142 A1 UPAB: 20060120
NOVELTY - Production of soyapogenol B from sophoradiol is by using a plant-originated hydroxylase.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a similar method for producing soyasapogenol B by incubating sophoradiol with the microsome fraction of cultured licorice cells in the presence of nicotinamide adenine dinucleotide phosphatase (NADPH).
ACTIVITY - Anticoagulant; Cytostatic; Hepatotropic.
MECHANISM OF ACTION - None given in source material.
USE - The method is for the production of soyapogenol B for use in drugs and pharmaceutical raw materials in treating e.g. thrombosis and tumor or protecting liver.
ADVANTAGE - The hydroxylase hydrolyzes sophoradiol at its 24-position to give soyasapogenol B efficiently.

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(FILE 'HOME' ENTERED AT 15:54:33 ON 09 APR 2008)

FILE 'CAPLUS' ENTERED AT 15:55:43 ON 09 APR 2008
E HAYASHI HIROAKI/AU 25
L1 9 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN
E INOUE KENICHIRO/AU 25
L2 7 S (E3 OR E4 OR E5) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE
E HOSHINO MASATERU/AU 25
L3 1 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN
E SHIBUYA MASAAKI/AU 25
L4 25 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN
E EBIZUKA YUTAKA/AU 25
L5 29 S (E3) AND (SOPHORADIOL OR ("TRITERPENE HYDROXYLASE") OR AMYRIN
L6 32 S L1 OR L2 OR L3 OR L4 OR L5
L7 32 DUP REM L6 (0 DUPLICATES REMOVED)

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS, SCISEARCH' ENTERED AT 16:16:54 ON 09 APR 2008
L8 3865 S 24-HYDROXYLASE OR "TRITERPENE HYDROXYLASE" OR (24-POSITION AN
L9 3862 S 24-HYDROXYLASE OR "TRITERPENE HYDROXYLASE" OR (AMYRIN (W) HYD
L10 7 S L8 AND (AMYRIN OR SOPHORADIOL)
L11 3 DUP REM L10 (4 DUPLICATES REMOVED)
L12 2 S L11 NOT L7